August 4, 1946.

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Dear Sir:

As I suggested when I wrote to you and Dr. Bruner a year ago, in a request for reprints, the phenomenon of phase variation in Salmonella has long seemed to me to be one of the most interesting in genetics. On the basis of research now in press by Dr. E.L. Tatum and myself, it may now be feasible to attack the problems of inheritance in coliform bacteria by Mendelian methods. Since it will be some time before this work is in print, I will take the liberty later in this letter of summarizing that work. This letter is addressed to you and Dr. Bruner, on the basis of my reading of the Salmonella literature, in the hope that we can initiate a collaborative project on Salmonella genetics, or if you prefer, that we can stimulate you to apply some of the procedures that we have developed in E. coli.

Briefly, we have found in E. coli evidence, that we find difficult to refute, for the recombination of characters in mixed populations. The characters that were used were a) a series of X-ray and ultra-violet induced mutations for growth factor requirements and b) spontaneous mutations for resistance to specific phages. Biochemical mutants grown together give rise to wild type cells; in addition, new combinations of nutritional requirements and phage resistance are found. There is sufficient data at this time that we are fairly confident of the existence of a recombination process, but before the work is published in extenso we should like to accumulate a good deal more. Recombination would seem to imply a method of sexual reproduction, on which we have no evidence. Under cultural conditions so far used the process is rare, about 1 cell in a million being a recombination type, and we have had to use selective media to demonstrate them. So far only one coli strain has been successfully used; another which was non-motile being unproductive. The recombinations occurred between mutants of the same strain.

While these conclusions are in the broadest sense highly tentative, it is my feeling that they are well enough established to explore the possibilities in other organisms. The tabulation of naturally occurring antigenic types in Salmonella has always suggested to me a recombination process, whereby different antigens can be associated with each other in the very many divers permutations characteristic of the numerous 'species.' It seems to me that it would be very worthwhile to attempt the production of new types by the method sketched above. This would involve inducing (by radiation) nutritional mutations in different Salmonella strains, growth in mixed culture, plating on minimal media to select the nutritional recombinations, and examination of these 'wild type' cultures for antigenic variations. While we have considerable experience in obtaining and handling nutritional mutants, we are not at all adept or equipped to do antigen analyses, for which reason we hope that we can arrange to work together.

To illustrate what is proposed consider the flagellar antigens of S. typhi-murium and S. abortus bovis, which in my table are characterised as i..1,2,3 and b.. enx. We would obtain biochemical mutants in both these strains, let us say biotin-less and leucineless, respectively. They would be grown in mixed culture, and then plated to isolate colonies of nutritional recombinants, requiring neither biotin, nor leucine. Such colonies (which occur in E. coli, and we hope may occur in Salmonella) would be sent by us to you for examination of antigenic status. One might look for the occurrence of flagellar antigenic formulae i..e,n,x such as is found in bonamiensis or b..1,2,3 in abony.

My cursory study suggests that these strains might be the most suitable as a start. Such an analysis is required before one can begin to consider phase variation. If you are disposed to work cooperatively with us, I would suggest that you send us cultures of the organisms mentioned, in both phases. It would also be helpful to have a small amount of monospecific sera so that we might do some preliminary screening of what we sent back to you (if anything/ arose ). I hope that the cultures mentioned are only mildly pathogenic if at all; we are not a medical laboratory. If you should have any other suggestions as to which strains might best be used, please voice them.

It is possible that you would prefer to work on this problem in your own laboratory. If so please let us know, and we shall send you our publications as soon as they are available. The barrier of distance may be of some importance; if there is anyone of special competence in the Salmonella serology in this area, with whom you feel this problem might be more profitably discussed please let me know.

Lastly, I should be grateful to you and Dr. Bruner if you would send me reprints of your papers for my files. J. Bact 37:365, 1938; 42:467, 1941, and Proc. Soc. 41:223 1939 have been received, for which, thanks.

Very sincerely yours,

Joshua Lederberg.